8. Following the last wash, tap the inverted wells onto absorbent paper to remove the last of the wash solution.
9. Dispense **100 µL of Substrate** into each well.
10. Shake the plate gently. Incubate the wells for **10 minutes**.
11. Dispense **100 µL of Stop Solution** into each test well. Shake the plate rack gently to mix.
12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

ADDITIONAL DILUTION PROCEDURE

Further dilution is necessary for highly contaminated samples (greater than 100 ppb).

1. Prepare 16% Methanol solution by mixing 1 part of the extraction solution (80% Methanol) with 4 parts of distilled or deionized water. Mix well and store tightly sealed.
2. Dilute the final extract after filtration with this 16% Methanol as desired. Run the assay with this diluted extract.
3. Apply the dilution factor to calculate the concentration of Aflatoxin in the sample.

   If you mixed 1 part of extract with 1 part of 16% Methanol, the dilution factor is 2. Multiply the result by 2.

   If you mixed 1 part of extract with 4 parts of 16% Methanol, the dilution factor is 5. Multiply the result by 5.

CALCULATE RESULTS

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance's to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of aflatoxin greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (Y axis; LOGIT) versus the calibrator concentration (X axis; LOG) on LOG-LOGIT graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the Y axis is the concentration of the sample.

BEACON ANALYTICAL SYSTEMS, INC.

82 Industrial Park Road

Saco, ME 04072

Tel. (207) 571-4302

Fax (207) 602-6502

www.beaconkits.com

SAMPLE CALCULATIONS

Well Contents	OD		Mean OD	SD*	%RSD	%Bo**	Afb1 ppb
0 ppb	1.773	1.702	**1.738**	0.050	2.89		
2 ppb	1.32	1.312	**1.316**	0.006	0.43	**75.7%**	**1.9**
7.5 ppb	0.825	0.837	**0.831**	0.008	1.02	**47.8%**	**8.2**
25.0 ppb	0.464	0.454	**0.459**	0.007	1.54	**26.4%**	**25.4**
100 ppb	0.187	0.184	**0.186**	0.002	1.14	**10.7%**	**95.9**
sample	0.663	0.706	**0.685**	0.030	4.44	**39.4%**	**12.4**

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302.

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

Beacon Analytical Systems Inc.

Aflatoxin Plate Kit

Cat.# 20-0017

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Aflatoxin Plate Kit is a competitive ELISA for the quantitative analysis of aflatoxin in nuts, grain and grain products. The kit has been certified as a Performance Tested Method[sm] by the AOAC Research Institute for use in corn and peanuts.

USE PRINCIPLES

The Aflatoxin kit is a competitive enzyme-labeled immunoassay. Aflatoxin is extracted from a ground sample by shaking or blending with methanol/water. The extract is diluted then filtered and tested in the immunoassay. Aflatoxin-HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts. Aflatoxin antibody is then pipetted into the test wells to initiate the reaction. During the 10 minute incubation period, aflatoxin from the sample and aflatoxin-HRP enzyme conjugate compete for binding to aflatoxin antibody which, in turn, binds to the test well. Following this 10 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound toxin or enzyme-labeled toxin. A clear substrate is then added to the wells and any bound enzyme-toxin conjugate causes the conversion to a blue color. Following a 10 minute incubation, the reaction is stopped and amount of color in each well is read. The color of unknown samples is compared to the color of the calibrators and the Aflatoxin concentration of the samples is derived.

MATERIALS PROVIDED IN THE BEACON AFLATOXIN PLATE

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC (1 yr from date of manufacture).

1 Plate containing 12 test strips of 8 wells each, vacuum-packed in aluminized pouch with indicating desiccant.

5 vials, each containing 2 mL of Aflatoxin calibrators corresponding to 0, 2.0, 7.5, 25 and 100 µg/L (ppb) of Aflatoxin. (Note: Because of the 1:10 dilution of the grain sample in the extraction step, the calibrators actually contain 1/10th of the stated value. No further correction back to the concentration in the original grain sample is required.)

1 vial containing 8 mL of Aflatoxin-HRP Enzyme Conjugate.

1 vial containing 8 mL of Rabbit anti-Aflatoxin antibody.

1 vial containing 14 mL of Substrate.

1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)

1 Instructional Booklet

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Aflatoxin Plate Kit cannot differentiate between the various Aflatoxins, but detects their presence to differing degrees. The following table shows the % cross reactivity (CR) versus Aflatoxin B_1.

COMPOUND	% CR
AFLATOXIN B_2	8
AFLATOXIN G_1	24
AFLATOXIN G_2	6

SENSITIVITY

	LOD	LOQ
Corn	0.4 ppb	1.2 ppb
Peanuts	0.6 ppb	1.8 ppb

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
- Methanol, ACS grade.
- Graduated cylinder, 100 mL or larger.
- Glassware for sample extraction and extract collection.
- Filter paper (coffee filter).
- Fisher Scientific G6 or equivalent glass fiber filter.
- Pipette with disposable tips capable of dispensing 50 µL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter. (Stat Fax Model 303 Plus)
- Timer.

PRECAUTIONS

1. Each reagent is optimized for use in the Beacon Aflatoxin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Aflatoxin Plate Kits with different Lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
3. Do not use reagents after expiration date.
4. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
5. Aflatoxin is a very toxic substance. Dispose of all liquids in a plastic container containing household bleach (minimum 10%). All lab ware should be soaked for at least 1 hour in a 10% solution of household bleach. Avoid contact of skin and mucous membranes with reagents and sample extracts by wearing gloves and protective apparel. If exposure of skin and mucous membranes to liquids should occur, immediately flush with water.
6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
7. The intended user of this kit is a trained laboratory technician. Familiarity with ELISA is recommended. Please contact Beacon for technical support if you have any questions about the use of this kit.
8. The use of a multichannel pipette to dispense the generic reagents (Conjugate, Antibody, Substrate and Stop) is recommended when running 2 strips or more.

EXTRACTION SOLUTION PREPARATION

1. Carefully measure 20 mL of distilled or deionized water for each 100 mL being prepared and transfer to a clean glass container with tight-fitting lid.
2. Carefully measure 80 mL of Methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix completely. Store tightly sealed to minimize evaporation.

SAMPLE PREPARATION

Corn and other grains

1. Grind samples to pass a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.
2. Weigh 50 g ground sample and 5.0 g NaCl and transfer to a clean blender jar.
3. Add 100 mL of 80% Methanol/water to the jar.
4. Blend for 1 minute in a high-speed blender.
5. Filter through a paper filter (Coffee filter is recommended).
6. Dilute 5 mL of extract with 20 mL of water and mix thoroughly.
7. Filter through a glass fiber filter.

Peanut paste

1. Weigh 50 g sample and transfer to a clean blender jar or other appropriately sized container with tight fitting lid (250 mL).
2. Add 100 mL of 80% Methanol/water to the jar.
3. Blend for 1 minute in a high-speed blender.
4. Filter a minimum of 10 mL through a paper filter (paper coffee filter is recommended).
5. Dilute 5 mL of extract with 20 mL of water and mix thoroughly. This solution can be assayed without filtering.

ASSAY PROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Dispense **50 µL of Enzyme Conjugate** into each test well.
4. Using a pipette with disposable tips, add **50 µL of calibrators and samples** to the appropriate test wells. Be sure to use a clean pipet tip for each.
5. Dispense **50 µL of Antibody Solution** into each test well.
6. Shake the plate gently to mix contents, incubate the test wells for **10 minutes**.
7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with Laboratory quality distilled or deionized water and dump. Repeat 4X for a total of five washes.